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Research report

Persistent activation of select forebrain regions in aggressive, adolescent cocaine-treated hamsters

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Abstract

Hamsters repeatedly exposed to cocaine throughout adolescence display highly escalated offensive aggression compared to saline-treated littermates. The current study investigated whether adolescent cocaine exposure activated neurons in areas of hamster forebrain implicated in aggressive behavior by examining the expression of FOS, i.e., the protein product of the immediate early gene *c-fos* shown to be a reliably sensitive marker of neuronal activation. Adolescent cocaine-treated hamsters and saline-treated littermates were scored for offensive aggression and then sacrificed 1 day later and examined for the number of FOS immunoreactive (FOS-ir) cells in regions of the hamster forebrain important for aggression control. When compared with non-aggressive, saline-treated controls, aggressive cocaine-treated hamsters showed persistent increases in the number of FOS-ir cells in several aggression regions, including the anterior hypothalamus, nucleus circularis, lateral hypothalamus (i.e., the hypothalamic attack area), lateral septum, and medial and corticomedial amygdaloid nuclei. Conversely, aggressive cocaine-treated hamsters showed a significant decrease in FOS-ir cells in the medial supraoptic nucleus, bed nucleus of the stria terminalis, and central amygdala when compared with controls. However, no differences in FOS-ir cells were found in other areas implicated in aggression such as the paraventricular hypothalamic nucleus, or in a number of non-aggression areas. These results suggest that adolescent cocaine exposure may constitutively activate neurons in select forebrain areas critical for the regulation of aggression in hamsters. A model for how persistent activation of neurons in one of these brain regions (i.e., the hypothalamus) may facilitate the development of the aggressive phenotype in adolescent cocaine-exposed animals is presented.
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Keywords: Aggression; Cocaine; Adolescence; FOS; Neural activation

1. Introduction

In a number of prior reports, we have used sub-adult Syrian hamsters as an animal model to examine the link between chronic adolescent cocaine exposure and the behavioral neurobiology of offensive aggression [10,28,41,52–54]. Behavioral data from these studies showed that hamsters repeatedly exposed to low doses of cocaine (0.5 mg/kg/day) throughout adolescent development display highly escalated, adult forms of offensive aggression characterized by intense bouts of biting and attacking primarily directed towards the flanks, rump and ventrum of the intruder, as well as high amounts of upright offensive postures and lateral attacks toward the intruder. The finding that adolescent cocaine-treated hamsters demonstrated highly escalated, adult forms of offensive aggression in the absence of prior social interactions and established dominance cues suggested that chronic adolescent cocaine exposure stimulated aggression directly, perhaps by impacting the development

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of specific neural systems and/or the activity of select neuronal populations that regulate this behavior.

Indeed, recently we have shown that aggressive, adolescent cocaine-treated hamsters display marked alterations in the development/activity of a number of neural systems that regulate offensive aggression in select areas of the hamster forebrain important for aggression control. For instance, in hamsters, serotonin (5-HT) activity has been shown to inhibit adolescent cocaine-induced offensive aggression, and aggressive, adolescent cocaine-treated hamsters display significant decreases in serotonin afferent innervation to the anterior hypothalamus, lateral septum, bed nucleus of the stria terminalis, and medial amygdala [10]. These reductions are coincident with site-specific increases in the expression of select 5-HT receptor pools and the density of afferent fiber systems that facilitate aggressive responding. For instance, in hamsters, 5-HT-type-3 receptor agonism induces offensive aggression [54]. Antagonism of 5-HT-3 receptor signaling suppresses adolescent cocaine-induced offensive aggression [53], and aggressive, adolescent cocaine-treated hamsters display significant increases in 5-HT-3 receptors in the anterior hypothalamus, lateral septum and central amygdala [53]. Similarly, in mice and hamsters, increased aggressive behavior correlates with higher γ-aminobutyric acid (GABA) activity, levels and density of synaptic terminals containing glutamic acid decarboxylase-65 (i.e., the rate-limiting enzyme in the synthesis of GABA) [25,29,51,56]. Aggressive, adolescent cocaine-treated hamsters display significant increases in GAD_{65} containing afferent terminals in several aggression regions, including the anterior hypothalamus and the medial and central amygdaloid nuclei [52]. It is possible that, either together or alone, these adolescent cocaine-induced neurodevelopmental changes stimulate offensive aggression by permanently altering the activation patterns of neurons in these discrete forebrain regions. To date, however, it is unknown whether adolescent cocaine exposure activates neurons in these brain areas important for aggression control.

In the nervous system, activated neurons can be identified by the expression of FOS, the protein product of the proto-oncogene *c-fos* [27,30]. Using a variety of species, immunohistochemical analysis of FOS expression has proven to be a reliably useful tool for mapping cellular activation resulting from a diverse array of physiologic and behavioral stimuli, including agonistic interactions [6,7,11,40,55,57,59]. For instance, directly following an aggressive interaction, rats, mice, and hamsters each show a significant increase in the number of FOS-containing neurons in many of the aforementioned forebrain areas important for aggression control, including the lateral septum, bed nucleus of the stria terminalis, hypothalamus (i.e., the anterior- and lateral-nucleus), and amygdala (i.e., the medial-, corticomedial- and central-nucleus) [4,8,9,11,21,22,26,33,35,39,40,49,50,58]. Although FOS had been originally thought to have been expressed for only short periods (i.e., 15–90 min) following stimulus exposure [43,44], instances of persistent FOS expression representative of constitutive cellular activation have been observed following chronic physiologic and behavioral stimulation [13,40,42]. Given these findings, the present studies were conducted to investigate the hypothesis that chronic adolescent cocaine exposure would produce *lasting* increases in neuronal activity in forebrain areas important for aggression control. From a functional standpoint, increases in neuronal activation for extended periods of time in these areas may represent constitutive activation of the neural circuit(s) implicated in the aggressive response, heightening the aggressive response patterns of these animals. To determine whether adolescent cocaine exposed animals possessed *persistent* alterations in neuronal activation patterns in areas of the hamster forebrain important for aggression control we quantified FOS-expressing cells in these brain sites 1 day following the behavioral test to assess aggressive responding.

2. Methods

2.1. Animals

Pre-pubertal (P21–23) male hamsters were obtained from Harlan Sprague–Dawley Labs (Indianapolis, IN), individually housed in polycarbonate cages, and maintained at ambient room temperature on a reverse light:dark cycle of (14L:10D; lights on at 19:00 h). Food and water were provided ad libitum. For aggression testing, stimulus (intruder) males of equal size and weight to the experimental animals were obtained from Harlan Spraque–Dawley 1 week prior to the behavioral test, group housed at five animals/cage in large polycarbonate cages, and maintained as above to acclimate to the animal facility. All intruders were pre-screened for low aggression (i.e., disengage and evade) and submission (i.e., tail-up freeze, flee, and fly-away) 1 day prior to the aggression test to control for behavioral differences between stimulus animals, as previously described [28,53,54]. Animals displaying significantly low aggression and/or submissive postures were excluded from use in the behavioral assay.

2.2. Experimental treatment

P27 Syrian hamsters were weighed and randomly distributed into two groups ($n > 8$ animals/group). One group of animals ($n = 12$) received intraperitoneal (i.p.) injections of 0.5 mg/kg cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) dissolved in 0.9% saline for 30 consecutive days during adolescent development (P27–P57). This daily dose of cocaine has been shown previously to facilitate highly escalated aggressive responding in adolescent hamsters [10,28,53]. The second group of hamsters ($n = 8$) received i.p. injections of saline (1.0 ml/kg) alone. The day following the last injection (P58), animals were tested for offensive aggression using the resident-intruder paradigm, sacrificed 24 h later (i.e., on P59), and the brains removed and processed for FOS immunohistochemistry as detailed below.

2.3. Aggression testing

Animals were tested for aggressive behavior using the resident/intruder paradigm, a well-characterized and ethologically valid model of offensive aggression in golden hamsters [20,36]. For

this measure, a stimulus (intruder) male of similar size and weight was introduced into the home cage of experimental animals and the resident was scored for several general measures of offensive aggression (i.e., number of attacks, bites, and latency to first bite towards an intruder) as previously described [53,54]. Briefly, an attack was scored each time the resident animal would wildly pursue and then either: (1) lunge towards and/or (2) confine the intruder by upright and sideways threat; each generally followed by a direct attempt to bite the intruder's ventrum and/or flank. The latency to bite was defined as the period of time between the beginning of the behavioral test and the first bite-containing attack of the residents towards an intruder. In the case of no bites, latency was assigned the maximum time of the behavioral test (i.e., 600 s). Each aggression test lasted for 10 min and was scored by an independent observer uninformed as to the experimental treatment. No stimulus animal was used for more than one behavioral test, and all tests were performed during the first 4 h of the dark phase under dim red illumination and videotaped for behavioral verification of the findings.

2.4. Immunohistochemistry

One day following the behavioral test for aggression, cocaine and saline-treated hamsters ($n = 12$ and 8, respectively) were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine and the brains fixed by transcardial perfusion with 4% paraformaldehyde (50 wt./50 wt.). Brains were then cryoprotected in 30% sucrose in phosphate buffered saline (1 × PBS; 0.001 M KH_2PO_4, 0.01 M Na_2HPO_4, 0.137 M NaCl, 0.003 M KCl, pH 7.4) overnight at 4 °C. A consecutive series of 35 μm coronal brain sections from experimental and control animals were cut on a freezing rotary microtome, collected as free-floating sections in 1 × PBS, and then every third section was labeled for FOS in one standardized immunohistochemical run using a modification of an existing protocol [10]. Briefly, sections were pretreated with 0.3% hydrogen peroxide for 30 min, washed in PBSTx (1 × PBS/0.5% Triton X-100), and pre-incubated in 5% normal goat serum (NGS) in PBSTx for 90 min. Sections were then incubated in rabbit antiserum (1:2000) generated against FOS (Oncogene Sciences) in 5% NGS/PBSTx for two nights at 4 °C. After primary incubation, sections were incubated in secondary anti-rabbit followed by avidin–biotin complex (Vectastain ABC Elite Kit-rabbit, Burlingame, CA) and then labeled with diaminobenzidine (DAB, Vector Labs, Burlingame, CA). Sections were mounted on gelatin-coated slides, allowed to air dry, and dehydrated through a series of ethanol and xylene solutions. Then, slides were coverslipped using Cytoseal-60 mounting medium (VWR Scientific, West Chester, PA, USA). Representative slides with omission of primary and/or secondary antibodies were processed as above for control purposes.

2.5. Image analysis

The number of FOS immunoreactive (FOS-ir) cells was determined within specific brain areas using the BIOQUANT NOVA 5.0 computer-assisted microscopic image analysis software package as previously described [10,24,53]. The areas analyzed were selected based on data from previous studies implicating these regions in aggressive responding across numerous species and models of aggression, with the notable exception of the S1 somatosensory neocortex (S1), the dorsolateral striatum (i.e., caudate–putamen, CPu), and the core and shell regions of the nucleus accumbens (AcbC and AcbSh, respectively) included in the assay as non-aggression areas used for control purposes. The specific aggression areas examined included the anterior hypothalamus (AH), the medial and lateral divisions of the bed nucleus of the stria terminalis (BNST), the central amygdaloid nucleus (CeA), the cortico-medial amygdaloid nucleus (CoMeA), the lateral hypothalamus (LH), the dorsal, intermediate, and ventral parts of the lateral septal nucleus (LS), the medial amygdaloid nucleus (MeA), the medial supraoptic nucleus (mSON), the nucleus circularis (NC), and the paraventricular hypothalamic nucleus (PVN) (Fig. 1). Slides from each animal were coded by an experimenter unaware of the experimental conditions and BIOQUANT NOVA 5.0 image analysis software running on a Pentium III CSI Open PC computer (R&M Biometrics, Nashville, TN, USA) was utilized to identify the brain Region Of Interest (ROI). Specifically, with the aid of The Hamster Atlas [45], a standard computer-generated parcel was drawn to outline the entire ROI at low power (4×) using a Nikon E600 microscope. Each brain region was assigned a separate and distinct ROI parcel, formatted in size specifically for that brain area, with the notable exception of the S1 cortex, CPu, and the AcbC and AcbSh control regions where placement of a size appropriate parcel was not feasible. In cases where the delin-

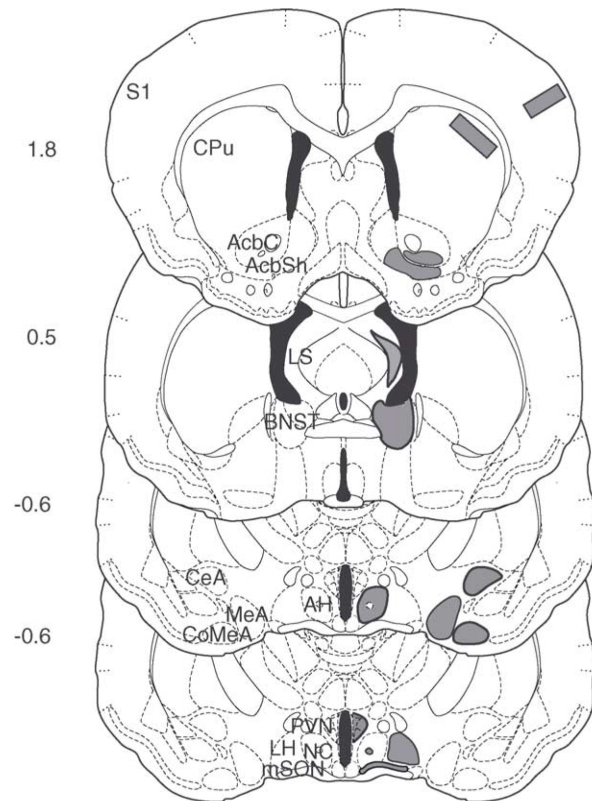

Fig. 1. Diagram showing the location of the areas selected to quantify FOS-containing cells (shaded areas). Plates were modified from hamster atlas of Morin and Wood (2001) [45] and reflect specific positions in the rostral-caudal plane (i.e., distance in mm from bregma to the plane of section at the skull surface). Abbreviations: AcbC, core region of the nucleus accumbens; AcbSh, shell region of the nucleus accumbens; AH, anterior hypothalamus; BNST, medial division of the bed nucleus of the stria terminalis; CeA, central amygdala; CoMeA, cortico-medial amygdala; CPu, dorsolateral part of the caudate–putamen; LH, lateral hypothalamus; LS, intermediate part of the lateral septal nucleus; MeA, medial amygdala; mSON, medial division of the supraoptic nucleus; NC, nucleus circularis; PVN, paraventricular hypothalamic nucleus; S1, somatosensory neocortex.

eation of ROI boundaries was questionable, extreme care was taken to localize nuclear compartments by measuring distances to standard neuronal landmarks including white matter fiber tracts, ventricular compartments and cortical borders. Then, under 10× magnification images were thresholded at a standard RGB-scale level empirically determined by observers unaware of the treatment conditions, such as to allow detection of stained FOS-ir cells with moderate to high intensity, while suppressing lightly stained elements. This threshold value was then applied across subjects to control for changes in background staining and differences in foreground staining intensity between animals. The illumination was kept constant for all measurements. FOS-ir cells were identified in each field using a mouse driven cursor and then FOS-ir counts were performed automatically by the BIOQUANT software. Measurements at 10× continued until FOS-ir elements throughout the entire ROI were quantified. Two to six independent measurements were taken from several consecutive sections ($n=2$–4) of each animal ($n=7$–11) per treatment group, with the exception of the AcbC and AcbSh where these small brain regions could only be identified in 3–4 animals/group. Then, the number of FOS-ir cells was determined for each ROI, averaged for each brain region per animal and used for statistical analysis.

2.6. Statistics

2.6.1. Behavioral studies

The results from the aggression tests were compared between cocaine- and saline-treatment groups. Nonparametric data (total number of attacks and bites) were compared by Mann–Whitney *U*-tests (two-tailed). Parametric data (latency to first bite) were compared by Student's *t*-test (two-tailed).

2.6.2. FOS immunoreactivity

The number of FOS-ir cells was compared between treatment groups by Student's *t*-test (two-tailed) for each area analyzed.

3. Results

3.1. Aggressive behavior

As characterized extensively in our previous studies [10,28,52–54], animals treated with low doses of cocaine hydrochloride during adolescent development showed significantly heightened measures of offensive aggression (Fig. 2). Hamsters treated with cocaine showed a significant increase in the number of attacks ($Z=3.39$, $p<0.001$) and bites ($Z=3.63$, $p<0.001$) directed toward the intruder. The majority of the cocaine-treated animals (7 out of 11) scored greater than 15 attacks during the aggression test. By comparison, most of saline-treated hamsters (six out of eight) scored less than two attacks or less on opponents. Similarly most of the cocaine-treated animals bit more during the behavioral test, with 9 out of 11 directing greater than 10 bites towards intruders, compared to saline-treated littermates where only one out of eight animals bit more than twice during the test. In addition, cocaine-treated hamsters displayed significantly decreased bite latencies toward intruders ($t(12)=7.4$, $p<0.001$), than vehicle-treated control animals. All but one cocaine-treated animal bit within the first 3 min of the 10-min test, in comparison to saline-treated control animals whose first recorded bite-containing attack averaged nearly 6 min later, toward the end of the test period.

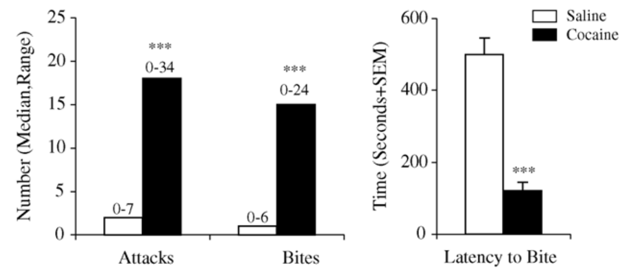

Fig. 2. Adolescent cocaine treatment increases offensive aggression. Number of upright offensive attacks and bites; as well as latency to first bite in cocaine- and saline-treated residents. Bars denote S.E.M. $^{***}p<0.001$; Mann–Whitney, two-tailed (number measures), Student's *t*-test, two-tailed (latency measure).

3.2. FOS immunohistochemistry

In aggressive, adolescent cocaine-treated hamsters, the immunohistochemical-staining pattern of FOS-containing

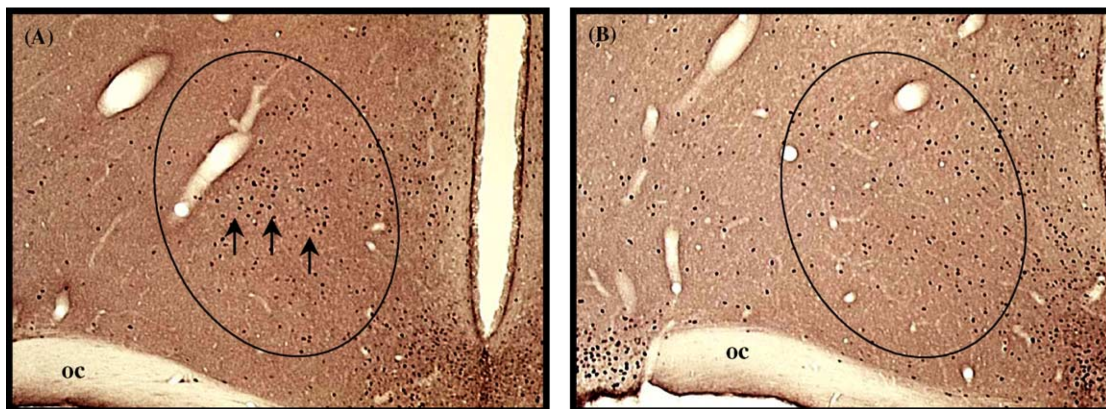

Fig. 3. Brightfield photomicrographs of a coronal section through the Syrian hamster hypothalamus. Shown are FOS-containing cells (arrows) within the anterior hypothalamus (encircled) of (A) cocaine-treated and (B) saline-treated hamsters. oc, Optic chiasm; 3 V, third ventricle.

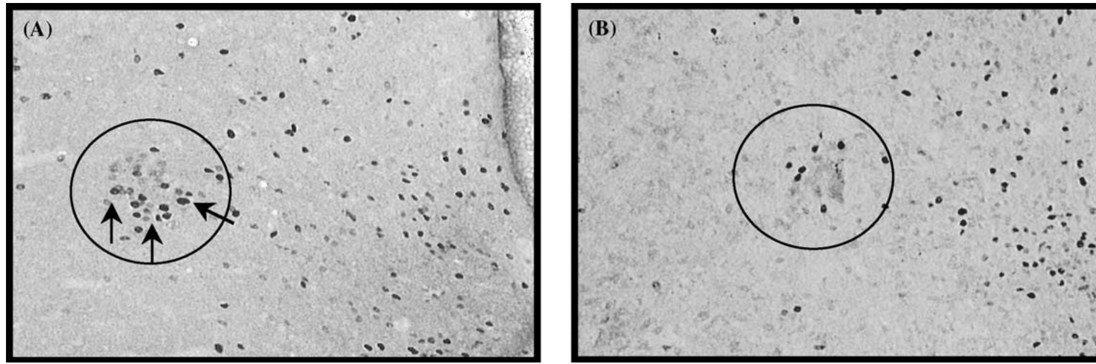

Fig. 4. Brightfield photomicrographs of a coronal section through the Syrian hamster hypothalamus. Shown are FOS-containing cells (arrows) within the nucleus circularis (encircled) of (A) cocaine-treated and (B) saline-treated hamsters.

cells was altered in several areas of hamster brain important for aggressive behavior, including those in the hypothalamus. For example, cocaine-treated hamsters exhibited increased number of FOS positive cells in the AH (Fig. 3A) when compared to saline-treated controls (Fig. 3B) which displayed a less dense pattern of staining, indicative of the normal distribution of FOS positive cells in this brain region. Quantitative analysis of FOS-ir cells in the AH showed that cocaine-treated animals had a two-fold increase in FOS positive cells when compared to saline-treated littermates (Fig. 5). This difference was statistically significant (AH, $t(13) = 12.9$, $p < 0.001$). Similar results were observed in the NC where aggressive, adolescent cocaine-treated animals had nearly four times the number of FOS positive cells than saline-treated littermates (Figs. 4 and 5). This difference was statistically significant (NC, $t(12) = 6.83$, $p < 0.001$). Also, aggressive, cocaine-treated hamsters displayed an increased number of FOS positive cells in the LH when compared to saline-treated controls (Fig. 5). This difference was statistically significant (LH, $t(11) = 2.34$, $p < 0.05$). These findings were not restricted to the hypothalamus, however, as other brain regions implicated in the hamster aggression circuit showed increases in the number of FOS positive cells following adolescent cocaine exposure (Fig. 5). For instance, the number of FOS positive cells in the LS of cocaine-treated animals was nearly two times that of saline controls and this difference was statistically significant (LS, $t(16) = 2.15$, $p < 0.05$). In addition, aggressive, adolescent cocaine-treated animals displayed an increased number of FOS positive cells in regions of the amygdala important for aggression. For instance, the number of FOS positive cells in the MeA and the CoMeA of cocaine-treated animals was nearly 1.5–2 times that of saline controls and this difference was statistically significant (MeA, $t(16) = 2.81$, $p < 0.05$; CoMeA, $t(10) = 4.02$, $p < 0.001$). However, aggressive, cocaine-treated animals did not show an increase in FOS positive cells in all brain regions implicated in the control of offensive aggression in hamster. For example, the number of FOS-containing cells in the mSON, BNST, and CeA were significantly decreased in cocaine-treated hamsters when compared to non-aggressive, saline-treated littermates (mSON, $t(7) = 4.48$, $p < 0.05$; BNST, $t(11) = 6.93$, $p < 0.001$; CeA, $t(8) = 6.04$, $p < 0.01$).

Not every brain region implicated in aggressive behavior in hamsters showed significant changes in the number of FOS positive cells following adolescent cocaine exposure. For instance, similar numbers of FOS-containing cells were found in the PVN of both cocaine-treated and control animals (Fig. 5). Similarly, no significant differences were found between treatment groups ($p > 0.05$ each comparison) in the S1 cortex, AcbC, AcbSh, nor CPu (Fig. 5), i.e., brain areas not involved in aggressive behavior in the hamster.

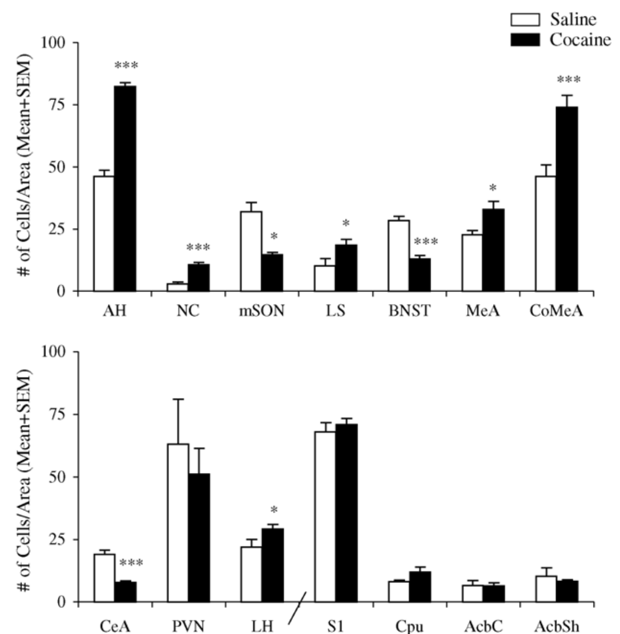

Fig. 5. Number of FOS-immunoreactive cells in select brain regions of cocaine- vs. saline-treated hamsters. $^{***}p < 0.001$, $^{*}p < 0.05$; Student's t-test, two-tailed.

4. Discussion

As previously reported in a number of studies, adolescent cocaine-treated animals showed highly elevated levels of offensive aggression when compared to saline-treated littermates (see Fig. 2 and Refs. [10,28,52–54]). Brains of aggressive, adolescent cocaine-treated animals showed persistent changes in the number of FOS-expressing neurons located throughout several key brain sites involved in aggression control in hamsters, including select sites within the hypothalamus, septum, and amygdala. More specifically, compared to non-aggressive, saline-treated controls, aggressive, adolescent cocaine-treated residents showed significant increases in the number of FOS-containing neurons in the AH, NC, LH, LS, MeA, and CoMeA suggesting a sustained increase in the activity of neurons in these brain regions. The finding that FOS-containing neurons are increased in these select regions of the hamster brain following an aggressive encounter is, in general, not unique. However, the findings that *persistent* increases in the number of FOS-expressing neurons occur in these brain sites in *adolescent cocaine-treated hamsters* are novel, and suggest a direct activational mechanism underlying the escalated levels of offensive aggression displayed in these animals during the behavioral test. Indeed, FOS has been originally thought to been expressed for only short periods (i.e., 15–90 min) following stimulus exposure [43,44]. Recent studies, however, have shown persistent FOS expression representative of constitutive cellular activation in response to chronic physiologic [13,42] and behavioral [13,40] stimuli. Thus, FOS appears to be expressed for substantial periods in brain cells following the appropriate stimuli. From a functional standpoint, the sustained increase in neuronal activation observed in areas of the hamster brain important for aggressive control (as indicated by an increased number of FOS-containing cells in these regions) in adolescent cocaine-treated animals may represent constitutive stimulation of the neural circuit(s) implicated in the aggressive response.

For instance, persistent activation of neurons in the AH (including the NC) may function to stimulate aggressive responding. Indeed, in hamster the AH is believed to be at the center of a neural network involved in control of offensive aggression [11]. In this brain region, arginine vasopressin (AVP) and serotonin neural signaling have been identified as two important neural systems controlling the aggressive state [16,18,19]. AH AVP activity in this brain region has been implicated in the facilitation of aggression, whereas AH 5-HT activity has the opposite effect, as it inhibits aggressive behavior (Fig. 6, first panel for model) [16,18]. The primary source of AVP afferent innervation to the AH appears to be magnocellular neurons located in the NC and the mSON [15,17,38]. AH AVP release has been demonstrated to modulate the activity of AH AVP neurons [23,60], indicating a receptor-mediated positive feedback action of endogenous AVP on its own release in this brain area [60]. A persistent increase in the activation of AVP neurons in the NC resulting from adolescent cocaine exposure may function to activate AH AVP neurons facilitating aggression as well as other non-AVP, aggression-stimulating neurons in this brain region (as well as the LH, i.e., a major site of AVP receptor expression within the hypothalamus [12]), augmenting the aggressive response pattern of adolescent cocaine-treated animals (Fig. 6, second panel). Indeed, recently we have shown that aggressive, adolescent cocaine-treated animals release more AVP into the AH than non-aggressive, saline-treated controls [5,31]. Further support for this notion can be found from experiments examining FOS and AVP co-localization in the AH (i.e., the NC and mSON). In preliminary studies, we have localized FOS expression to AVP neurons of the NC using FOS/AVP double label immunohistochemistry under basal and stimulated test conditions in cocaine-treated animals only compared to saline controls (data not shown). In hamsters trained to be highly aggressive, AVP neurons in the NC and mSON showed increased activity (i.e., number of AVP/FOS-containing neurons) in aggressive, trained fighters, supporting their role in the control of offensive aggression [11]. The lack of an increase in the activation of neurons in the mSON following adolescent cocaine-exposure seen in the current study may indicate that neurons in the NC are more critical for the escalated behavioral response in this model. This hypothesis is currently under investigation in the laboratory.

One putative mechanism that might explain the increase in number of FOS-ir cells in the AH and NC (and not the mSON) of aggressive, adolescent cocaine-treated animals stems from findings regarding 5-HT inhibition of AH (and NC) neurons facilitating the aggressive response. A growing body of neurobiological and behavioral data indicates that 5-HT inhibits aggression by suppressing the activity of AH AVP. Anatomical studies reveal a dense 5-HT afferent innervation originating from neurons in the dorsal and median raphe nucleus onto AH AVP neurons, most notably those in the NC [18,19]. Functionally, treatment of hamsters with fluoxetine (a selective serotonin reuptake inhibitor that increases the available extracellular pools of 5-HT) increases AH 5-HT [46], decreases AH AVP release [1,14], and reverses aggression resulting from application of AVP directly into the AH [14,18]. Similarly, direct AH microinjections of select 5-HT receptor agonists reverses aggression resulting from the introduction of AVP into the AH [19]. Might a loss of 5-HT inhibition onto AH and NC neurons (the latter of which is positioned as a small cluster of neurons centered directly within the AH proper (Fig. 1)) explain the persistent activation of cells in these brain regions? Recently, we have shown a dramatic reduction in 5-HT-containing afferents to the AH and NC of aggressive, adolescent cocaine-treated hamsters [10]. Aggressive, adolescent cocaine-treated animals had only about half of the 5-HT afferent development to the AH of non-aggressive, saline-treated littermates, indicating a markedly reduced 5-HT tone in this brain region in cocaine-exposed animals. Lending further credibility to this hypothesized mechanism of action are data from

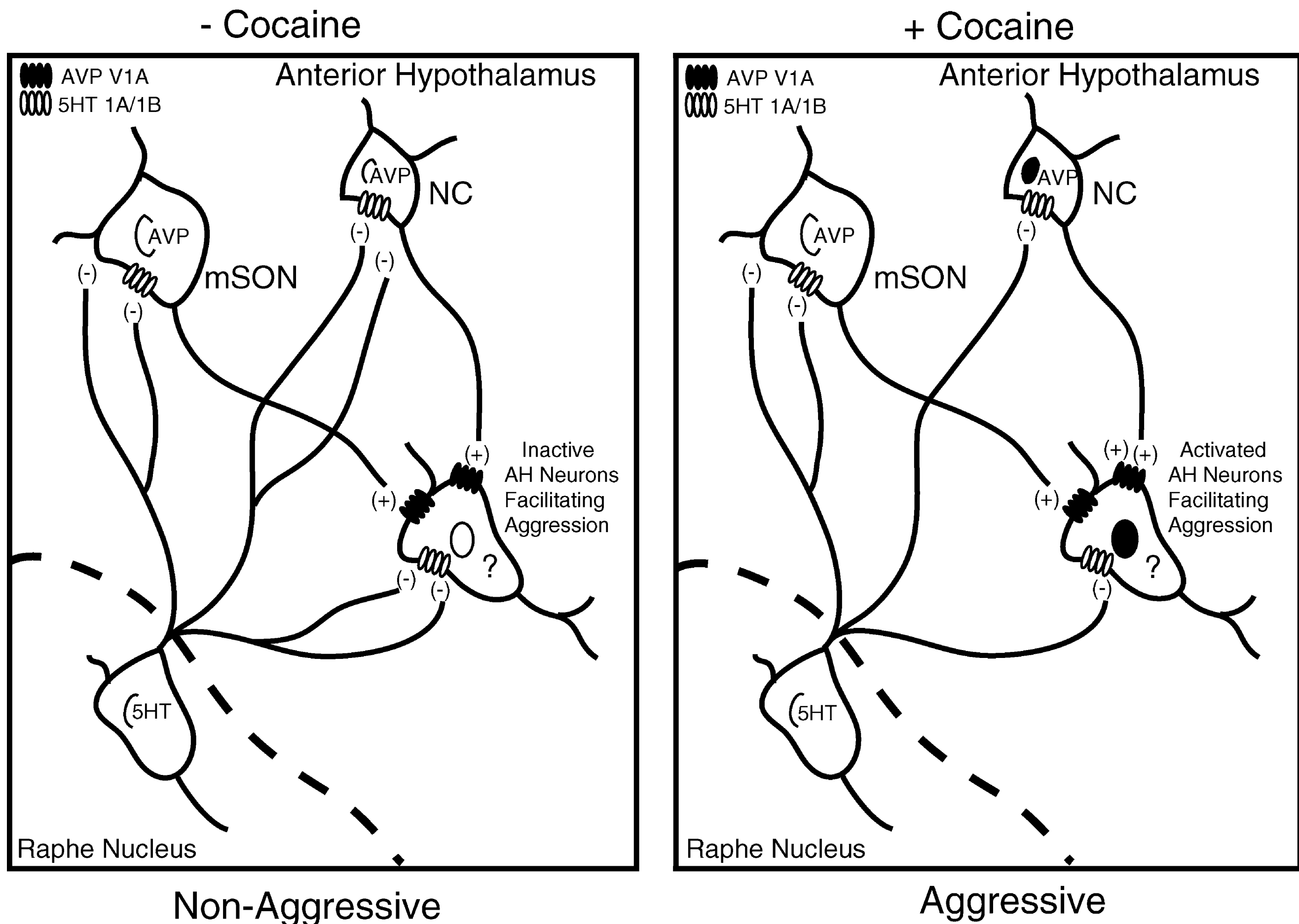

Fig. 6. A model showing the hypothetical relationship between adolescent cocaine-induced neuronal activation and the serotonin (5-HT) with the arginine vasopressin (AVP) systems in the AH (adapted from [18]). In the first panel (-cocaine) a dense plexus of 5-HT afferent fibers originating from neurons in the raphe nucleus innervate AVP neurons localized to the medial supraoptic nucleus (mSON) and nucleus circularis (NC), i.e., vasopressinergic neurons identified as potential sources of AVP innervation to the AH involved in agonistic behavior. These AVP neurons together with 5-HT neurons from the raphe nucleus regulate the activity of AH neurons involved in the facilitation of aggression. The identity (*?*) of these post-synaptic AH neurons is unknown. 5-HT is inhibitory (−), working through a 5-HT1A/B receptors, whereas AVP is excitatory (+), working through a V1A receptor. In the second panel (+cocaine), adolescent cocaine exposure markedly reduces 5-HT afferent innervation to the AH (and NC) (see Ref. [10]), constitutively dis-inhibiting (i.e., activating) AH vasopressinergic- and non-vasopressinergic-neurons facilitating aggression (neuronal activation represented as dark nuclei indicating persistent FOS expression).

studies examining FOS expression in mice and rats with decreased 5-HT tone. BDNF knockout mice that exhibit blunted 5-HT development display increases in the number of FOS-containing neurons in brain that can be reduced by treatment with fluoxetine [37]. Similarly, 5-HT depleted rats that undergo repeated social interactions show increases in the number of FOS containing neurons in brain [8]. Thus, decreases in 5-HT tone appear to activate downstream neurons. In the case of aggressive, adolescent cocaine-treated animals, the blunted 5-HT development/tone that exists in the AH as a result of cocaine exposure might chronically activate downstream neurons in this brain region that facilitate the aggressive response, including AVP neurons in the NC and non-AVP neurons in the AH proper (Fig. 6, second panel).

This biological assertion may be applied to extrahypothalamic brain sites as well. For example, aggressive, adolescent cocaine-treated animals show significant increases in FOS-containing neurons in the LS, MeA and CoMeA. In each of these brain regions, significant reductions (>30%) in 5-HT afferent innervation have been shown [10]. And as mentioned above, offensive aggression has been shown to be activated by stimulation of cells located in the CoMeA [50], and an increase in FOS-containing neurons in the LS, MeA and CoMeA have been detected following an aggressive encounter with other hamsters [11,35,49]. Thus, the blunted 5-HT development/tone that exists in these brain areas as a result of cocaine exposure might constitutively activate downstream neurons that facilitate aggressive behavior. These data notwithstanding, there are several findings from the current study that are inconsistent with the general hypothesis that adolescent cocaine activates neurons (and brain regions) implicated in aggression facilitation. For instance,

in this study we show marked activation of neurons in the LS in aggressive, adolescent cocaine-treated animals. However, intraspecific aggression has been shown to be inhibited by activation of the LS in hamsters [47,48]. Also, our previous study showed a significantly reduced 5-HTergic afferent innervation to the BNST in aggressive, cocaine-treated hamsters compared to saline-treated controls. Thus, under our hypothesis, an increase in the number of FOS-containing neurons in the BNST of aggressive, cocaine-treated animals should have been observed when compared to controls. However, aggressive, cocaine-treated hamsters showed a *decreased* number of FOS-expressing cells compared to non-aggressive, saline-treated controls. While it is true that the LS and BNST have previously been reported to be active following bouts of agonistic behavior in hamsters [33,35], these brain regions have not been specifically characterized as regulating offensive forms of aggressive behavior in hamsters. For instance, in hamsters, activity and/or activation of the LS and BNST has been implicated in defensive behavior, mating and scent marking [2,3,32–34]. Therefore, activity in these brain regions may have less of an affect on purely *offensive responding* observed in the adolescent cocaine model, remaining consistent with our central hypothesis regarding the site-specific nature of neural activation and the development of the offensive aggressive phenotype. Taken together, these data above are novel and significant in that they show that chronic exposure to low-dose cocaine during adolescent development can *persistently* alter patterns of neural activation in areas of the brain which have been implicated in aggression facilitation in hamsters. From a neurobiological standpoint, these data implicate increased neural signaling in several key areas as potential neural substrates for adolescent cocaine-induced offensive aggression. Further, the different patterns of neuronal activation across several "aggression regions" coupled with the unchanging and stable nature of neural activation across several "non-aggression" regions suggest that there is a non-uniform effect of chronic adolescent cocaine-treatment on neuronal activation across the neuraxis.

In summary, the neural mechanisms underlying the elicitation of cocaine-induced offensive aggression appears to be complicated and likely involves a number of neurotransmitter systems, as indicated by our previous work showing that aggressive, adolescent cocaine-treated animals have region-specific altered 5-HT, AVP and GABA neural systems. That notwithstanding, the studies presented in this paper provide data regarding the neurobiological effects of chronic low-dose cocaine exposure during adolescent development and propose a basic neurobiological mechanism by which these agents may exert their aggression-stimulating effects. These findings indicate that chronic low-dose cocaine exposure throughout adolescent development increases the activation of neurons in several key areas of hamster brain important for the facilitation of aggressive behavior, including the AH, NC, LH, MeA, and CoMeA. These findings, together with those from our laboratory indicating that the 5-HT and AVP neural systems are important in adolescent cocaine-induced offensive aggression in hamsters, are synthesized to provide a putative neural mechanism explaining how adolescent cocaine exposure might facilitate aggressive responding. Further studies are needed to elucidate whether the aggression-stimulating effects of cocaine exposure do indeed occur as a direct result of cocaine's influence on the signaling between these systems or whether the changes in neural activation observed here were representative of activational changes in other endogenous neural systems.

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